Tumour Infiltrating (TINKs) and Tumour Associated (TANKs) Natural Killer cells in colorectal cancer: a new paradigm in the inflammatory angiogenic switch

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Colon cancer is often associated with inflammation. We previously found that the innate immunity activatory molecule TRL4 is a negative prognostic factor in colon cancer [1]. As a consequence of their plasticity, immune cells are able to acquire altered phenotype/functions in cancer patients that include impaired cytotoxicity, acquisition of an immunosuppressive behaviour and of pro-angiogenic functions. The role of natural killer cells (NK), cellular mediators of the innate immunity, primarily involved in tumour recognition, to cancer promotion/progression still represents an unexplored topic. Altered NK phenotype and functions have been observed in different tumours. We demonstrated that tumour infiltrating (TINK) and tumour associated (TANK, i.e peripheral blood) NK cells are switched to a highly pro angiogenic phenotype in patients with non small cell lung cancer (NSCLC) and that TGFb acts as one inducer of this process. We are now studying the phenotype and function of NK cells from patients with colorectal cancer (CRC) and investigating their contribution to tumour angiogenesis [2,3].

NK subset distribution and cytokine profiling were performed by multicolor flow cytometry, using peripheral blood and tissue samples from CRC patients, for surface antigen and cytokine profiling characterization. Conditioned media (CM) from FACS-sorted NKs were used either for secretomic profiling, using antibody membrane array or in functional *in vitro* angiogenesis assays.

We found that $CD56^{bright}CD16^{-}$ NK cells predominate in CRC adjacent and tumor tissues. They produce VEGF, PlGF, IL-8 and show impaired cytotoxicity. Further, TINK/TANKs from CRC patients express the decidual NK markers CD9 and CD49a. Secretomic and FACS analysis on CRC peripheral blood NKs revealed up regulation for several pro angiogenic factors, including Angiogenin, Angiopoietin-1/2, TIMP-1/2, Tie-2, MMP1, MMP9. CM of FACS sorted NK cells from peripheral blood and tumor tissue of CRC patients were able to induce HUVEC proliferation, migration, adhesion and the formation of capillary like structures.

Our data demonstrate that TINK/TANKS from CRC patients are switched toward a pro-angiogenic/pro-tumor phenotype and function. We propose that TINK/TANKs could represent a new hallmark in CRC inflammation.

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